C.F.R. §1.821(c) as new pages 1-26. Furthermore, attached hereto is a 3 1/2" disk containing the "Sequence Listing" in computer readable form in accordance with 37 C.F.R. §1.821(e).

Applicants have amended the specification to insert SEQ ID Nos, as supported in the present specification.

The following statement is provided to meet the requirements of 37 C.F.R. §1.825(a) and 1.825(b).

I hereby state, in accordance with 37 C.F.R. §1.825(a), that the amendments included in the substitute sheets of the sequence listing are believed to be supported in the application as filed and that the substitute sheets of the sequence listing are not believed to include new matter.

I hereby further state, in accordance with 37 C.F.R. §1.825(b), that the attached copy of the computer readable form is the same as the attached substitute paper copy of the sequence listing.

Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that

organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Applicants submit that the present application contains patentable subject matter and therefore urge the examiner to pass the case to issuance.

If the examiner has any questions or comments concerning the above described application, the examiner is urged to contact the undersigned at the phone number below.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning at the bottom of page 20, has been amended as follows:

This reaction product was admixed with 2.5-fold volumes of ethanol and 2 μl of 3 M sodium acetate, and allowed to stand at -20°C for 2 hours to precipitate the cDNA. The precipitate was collected, washed with 75%(v/v) ethanol in water, dissolved in sterilized-distilled water, admixed with 0.5 μl of 2.5 units/μl DNA polymerase ("Cloned Pfu polymerase," product of Stratagene), 10 μl of 25 mM dNTP mix, and further admixed with the oligonucleotide shown by 5'-ACNCCNGTNWSNCA-3' (SEQ ID NO:52) as a sense primer, chemically synthesized on the basis of the amino acid sequence of SEQ ID NO:3, and the oligonucleotide shown by 5'-TGNGCNARNACNACRTG-3' (SEQ ID NO:53) as an antisense primer, chemically synthesized on the basis of the amino acid sequence of SEQ ID NO:8, both in a volume of 10 μM, and the total volume was adjusted to 100 μl with sterilized-distilled water. This mixture was incubated under 40 cycles of the sequential conditions at 94°C, 40°C, and 72°C for 1 minute each to effect PCT.

The paragraph beginning at page 21, line 7, has been amended as follows:

A portion of the PCR product was collected and then electrophoresed on 1%(w/v) agarose gel to separate DNA fragments, and the DNA fragments were transferred to a nylon

membrane and fixed thereon with 0.4 N sodium hydroxide. The membrane was washed with 2 x SSC, dried in air, immersed in prehybridization solution containing 6 x SSPE, 5 x Denhardt's solution, 0.5%(w/v) SDS, and 100 μg/ml denatured salmon sperm DNA, and incubated at 65°C for hours. A probe was prepared by chemical synthesis of the oligonucleotide shown by 5'-GGRCANGGRTCYTT-3' (SEQ ID NO:54), based on the amino acid sequence shown in SEW ID NO:3, and isotope-labeling thereof with [γ-^{32}P]ATP by T4 polynucleotide kinase. To the pre-hybridization solution in which the above nylon membrane had been immersed, 1 pmol of the probe was added, and the nylon membrane was incubated at 40°C for another 20 hours to effect hybridization. The nylon membrane was washed with 6 x SSC and subjected to autoradiography in a usual manner. A specific hybridization signal by the nylon membrane was incubated at 40°C for another 20 hours to effect hybridization. The nylon membrane was washed with 6 x SSC and subjected to autoradiography in a usual manner. A specific hybridization signal by the probe was observed. This showed that the above PCR product contained the objective DNA fragment.

The paragraph bridging pages 21 and 22 has been amended as follows:

To the remaining part of the above PCR product, 1 ng of a plasmid vector ("pCR-Script Cam SK(+)," produced by Stratagene) was added, and the DNA fragment of the PCR product was inserted into the vector with a DNA ligation kit ("DNA

Ligation Kit, Version 2," produced by Takara Shuzo Co., Ltd.). With a portion of the reaction mixture collected, an *Escherichia coli* strain ("XL1-Blue MRF' Kan," produced by Stratagene) was transformed. The transformant was inoculated in LB medium (pH 7.5) containing 30 μg/ml chloramphenicol and cultured at 37°C for 18 hours. The cells were collected from the culture. The plasmid DNA was collected from the cells in a usual manner, and analyzed by dideoxy method. This plasmid DNA comprised the nucleotide sequence shown in SEQ ID NO:34 as the sequence of the DNA fragment produced by PCR. The amino acid sequence encoded by this nucleotide sequence, aligned therewith, were compared with the partial amino acid sequences determined in Examples 1-2 to 1-3, shown in SEQ ID NOs:3 to 23. These partial amino acid sequences were completely or partly included by the amino acid sequence (SEQ ID NO:42) aligned in SEQ ID NO:34. This suggested that the nucleotide sequence shown in SEQ ID NO:34 encodes at least a part of the IL-18-binding protein of human origin.

The paragraph beginning at the bottom of page 22, has been amended as follows:

Ten nanograms of human liver poly(A)$^+$ RNA (product of Clontech) was subjected to 5'RACE, a modified method of PCR, with a commercially available 5'RACE kit ("5'RACE System, Version 2.0," product of GIBCO BRL). First, reverse transcriptase reaction was effected on the above RNA with the oligonucleotide shown by 5'GGTCACTTCCAATGCTGGACA-3' (SEQ ID

NO:55) as a primer, chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:34, and to the 5'-terminal of the first strand cDNA synthesized thereby, C-tail was added by the action of terminal deoxynucleotidyl transferase. Then, PCR was effected on this first strand cDNA with the oligonucleotide shown by 5'-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3' (SEQ ID NO:56) as a sense primer, included by the above kit, and the oligonucleotide shown by 5'-GTCCTTTGTGCTTCTAACTGA-3' (SEQ ID NO:57) as an antisense primer, chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:34. A portion of the produce of this 5'RACE was collected, and electrophoresed in a usual manner on 1%(w/v) agarose. Specific amplification of a DNA fragment was observed. This DNA fragment was sequenced similarly as in Example 2-1(a). This fragment comprised the nucleotide sequence shown in SEQ ID NO:35. The sequence from the 160^{th} to 216^{th} nucleotides of this sequence completely matched with the sequence from the 1^{st} to 57^{th} nucleotides of the nucleotide sequence shown in SEQ ID NO:34, determined in Example 2-1(a). This suggested that the nucleotide sequence shown in SEQ ID NO:35 overlaps with the nucleotide shown in SEQ ID NO:34, encoding at least a part of the IL-18-binding protein (SEQ ID NO:43) of human origin, and comprises the 5'-upsteam region of SEQ ID NO:34.

The paragraph beginning at page 24, has been amended as follows:

Ten nanograms of human liver poly(A)$^+$ RNA was subjected to 3'RACE, a modified method of PCR, in accordance with "PCR Jikken Manual (Manual for PCR Experiments)," transplanted by Takashi Saito, published by HBJ Press (1991), 25-33. First, reverse transcriptase reaction was effected on the above RNA with the oligonucleotide shown by 5'-GACTCGAGTCGACATCGA$(T)_{17}$-3' (SEQ ID NO:58) as a primer. Then, PCR was effected on the first strand cDNA synthesized thereby with the oligonucleotide shown by 5'-TTCTCCTGTGTGCTCGTGGA-3' (SEQ ID NO:59) as a sense primer, chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:34, determined in Example 2-1(a), and the oligonucleotide shown by 5'-GACTCGAGTCGACATCG-3' (SEQ ID NO:60) as an antisense primer. A portion of the product of this 3'RACE was collected and electrophoresed in a usual manner on 1%(w/v) agarose. Specific amplification of a DNA fragment was observed. This DNA fragment was sequenced similarly as in Example 2-1(a). This fragment comprised the nucleotide sequence shown in SEQ ID NO:36. The sequence from the 1st to 60th nucleotides of this sequence completely matched with the sequence from the 352nd to 411st nucleotides of the nucleotide sequence shown in SEQ ID NO:34, determined in Example 2-1(a). This suggested that the nucleotide sequence shown in SEQ ID NO:36 overlaps with the nucleotide sequence shown in SEQ ID NO:34, encoding at least a part of the IL-18-binding protein (SEQ ID NO:44) of human origin, and comprises the 3'-downstream region of SEQ ID NO:34.

The two paragraphs beginning at page 25, line 11, and ending at page 26, line 20, have been amended as follows:

In accordance with the method in Example 2-1(a), reverse transcriptase reaction was effected on human liver poly(A)$^+$ RNA, and then PCR was effected similarly as in Example 2-1(a) except for using as a sense primer the oligonucleotide shown by 5'-TGTGTGACTGGAGAAGAGGAC-3' (SEQ ID NO:50), chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:37, and as an antisense primer the oligonucleotide shown by 5'-TACAGGCAGTCAGGGACTGTTCACTCCAG-3' (SEQ ID NO:51), chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:37. A portion of the PCR product was collected, and electrophoresed in a usual manner on 1%(w/v) agarose gel. Specific amplification of a DNA fragment was observed. This DNA fragment was sequenced similarly as in Example 2-1(a). This fragment comprised the nucleotide sequence shown in SEQ ID NO:37. This supported that the nucleotide sequences shown in SEQ ID NOs:34 to 36, determined in Examples 2-1(a) to 2-1(c), are partial sequences of the contiguous nucleotide sequence shown in SEQ ID NO:37.

The amino acid sequence (SEQ ID NO:45) encoded by the nucleotide sequence shown in SEQ ID NO:37, aligned therewith, are compared with the partial amino acid sequences shown in SEQ ID NOs:4 to 23, determined in Example 1-3. These partial sequences were all included by the amino acid sequence aligned in SEQ ID NO:37 in the region from the 1st to 164th amino acids. In addition, the N-terminal amino acid sequence

determined in Example 1-2, shown in SEQ ID NO:3, well matched with the amino acid sequence aligned in SEQ ID NO:37 in the region from the 1st to 22nd amino acids. These facts suggested that the nucleotide sequence shown in SEQ ID NO:37 can encode the IL-18-binding protein of human origin by the region from the 160th to 651st nucleotides and that this IL-18-binding protein may has, as its whole sequence, the sequence from the 1st to 164th amino acids of the amino acid sequence aligned with this nucleotide sequence. Thus suggested amino acid sequence of the IL-18-binding protein of human origin and the nucleotide sequence encoding this are shown in SEQ ID NOs:1 and 32 separately.

The paragraph at the bottom of page 26, has been amended as follows:

A DNA capable of encoding the IL-18-binding protein of human origin, obtained by the method in Example 2-1(d), was placed in a 0.5-ml reaction tube in an amount of 1 ng, and to this tube, 10 µl of 10 x PCR buffer, 1 µl of 25 mM dNTP mix, and 2.5 units/µl DNA polymerase ("Cloned Pfu polymerase," produced by Stratagene) were added. Appropriate amounts of the oligonucleotide shown by 5'-CTCGAGGCCACCATGACCATGAGACACAAC-3' (SEQ ID NO:61) as a sense primer, chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:32, and the oligonucleotide shown by 5'-GCGGCCGCTCATTAGTGATGGTGATGGTGATGACCCTGCTGCTGTGGACT-3' (SEQ ID NO:62) as an antisense primer, chemically synthesized on the

basis of the nucleotide sequence shown in SEQ ID NO:32, were further added to the above tube, and the total volume was adjusted to 100 µl with sterilized-distilled water. PCR was effected by incubating this mixture under 3 cycles of the sequential conditions at 94°C for I minute, at 42°C for 2 minutes, and at 72°C for 3 minutes and then 35 cycles of the sequential conditions at 94°C for 1 minute, at 60°C for 2 minutes, and 72°C for 3 minutes. The PCR product was analyzed and manipulated similarly as in Example 2-1(a); the PCR product was confirmed to contain the objective DNA fragment, and a plasmid vector inserted with this DNA fragment was obtained. This plasmid DNA comprised the nucleotide sequence shown in SEQ ID NO:32, confirmed by sequencing similarly as in Example 2-1(a).

The paragraph beginning at page 33, line 14, has been amended as follows:

Reverse transcriptase reaction was effected similarly as *in* Example 2-1(a) on this total RNA, and PCR was effected on this reaction product containing first strand cDNA similarly as in Example 2-1(a) except for using as a sense primer the oligonucleotide shown by 5'-GCNGTNCCNACNAA-3' (SEQ ID NO:63), chemically synthesized on the basis of the amino acid sequence shown in SEQ ID NO: 27, and as an antisense primer the oligonucleotide shown by 5'-GTYTTNARNCCRTC-3' (SEQ ID NO:64), chemically synthesized on the basis of the amino acid sequence shown in SEQ ID NO:30. A probe was prepared from the oligonucleotide shown by 5'-SWNCTRTGNCCYTCYTT-3' (SEQ ID

NO:65), chemically synthesized on the basis of the amino acid sequence shown in SEQ ID NO:24. By using this probe and by the procedure according to Example 2, the above PCR product was confirmed to contain the objective DNA fragment. This DNA fragment was sequenced similarly as in Example 2-1(a). This fragment comprised the nucleotide sequence shown in SEQ ID NO:38. The amino acid sequence (SEQ ID NO:46) aligned in SEQ ID NO:38 was compared with the partial amino acid sequences shown in SEQ ID NOs:24 to 31, determined in Example 3-2. These partial amino acid sequences were completely or partly included by the amino acid sequence aligned in SEQ ID NO:38. This suggested that the nucleotide sequence shown in SEQ ID NO:38 encodes at least a part of the IL-18-binding protein of mouse origin.The paragraph beginning on page 34, line 14 has been amended as follows:

Total RNA was collected similarly as in Example 4-1(a) from female CD-1 mice treated with the dead cells of *Corynebacterium parvum* and lipopolysaccharide, and 1 μg of the total RNA was subjected to 5'RACE, a modified method of PCR, with a commercially available 5'RACE kit ("5'RACE System, Version 2.0," product of GIBCO BRL). First, reverse transcriptase reaction was effected on the above total RNA with the oligonucleotide shown by 5'-TGCAGGCAGTACAGGACAAGG-3' (SEQ ID NO:66) as a primer, chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:38, and to the 5'-terminal of the first strand cDNA synthesized thereby, C-tail was added by the action of terminal deoxynucleotidyl

transferase. Then, PCR was effected on this first strand cDNA with the oligonucleotide shown by 5'-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3' (SEQ ID NO:56) as a sense primer, included by the kit, and the oligonucleotide shown by 5'-GTGCTGGGTACTGCTTAGTTG-3' (SEQ ID NO:67) as an antisense primer. A portion of this 5'RACE product was collected, and electrophoresed in a usual manner on 1%(w/v) agarose gel. Specific amplification of a DNA fragment was observed. This DNA fragment was sequenced similarly as in Example 2-1(a). This fragment comprised the nucleotide sequence shown in SEQ ID NO:39. The sequence from the 307th to 336th nucleotides of this sequence completely matched with the sequence of the 1st to 30th nucleotides of the sequence shown in SEQ ID NO:38, determined in Example 4-1(a). This suggested that the nucleotide sequence shown in SEQ ID NO:39 overlaps with the nucleotide sequence shown in SEQ ID NO:38, encoding at least a part of the IL-18-binding protein (SEQ ID NO:47) of mouse origin, and comprises the 5'-upstream region of SEQ ID NO:38.

The paragraph beginning at page 35, line 19, has been amended as follows:

Total RNA was collected similarly as in Example 4-1(a) from female CD-1 mice treated with the dead cells of *Corynebacterium parvum* and lipopolysaccharide, and 1 μg of the total RNA was subjected to 3'RACE, a modified method of PCR, in accordance with the methods described in "PCR *Jjkkeri* Manual (Manual for PCR Experiments)," translated by Takashi

Saito, published by HBJ Press (1991), pp.25-33. First, reverse transcriptase reaction was effected on the above total RNA with the oligonucleotide shown by 5'-GACTCGAGTCGACATCGA$(T)_{17}$-3' (SEQ ID NO:58) as a primer. Then, PCR was effected on the first strand cDNA synthesized thereby with the oligonucleotide shown by 5'-GATCCTGGACAAGTGGCC-3' (SEQ ID NO:68) as a sense primer, chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:38, determined in Example 4-1(a), and the oligonucleotide shown by 5'-GACTCGAGTCGACATCG-3' (SEQ ID NO:60) as an antisense primer. A portion of this 3'RACE product was collected, and electrophoresed in a usual manner on 1%(w/v) agarose gel. Specific amplification of a DNA fragment was observed. This DNA fragment was sequenced similarly as in Example 2-1(a). This fragment comprised the nucleotide sequence shown in SEQ ID NO:40. The sequence from the 1st to 63rd nucleotides of this sequence completely matched with the sequence of the 289th to 351st nucleotides of the sequence shown in SEQ ID NO:38, determined in Example 4-1(a). This suggested that the nucleotide sequence shown in SEQ ID NO:40 overlaps with the nucleotide sequence shown in SEQ ID NO:38, encoding at least a part of the IL-18-binding protein (SEQ ID NO:48) of mouse origin, and comprises the 3'-downstream region of SEQ ID NO:38.

The paragraph beginning at page 37, line 3 has been amended as follows:

Total RNA was collected similarly as in Example 4-1(a) from female CD-1 mice treated with the dead cells of *Corynebacterium parvum* and lipopolysaccharide. After reverse transcriptase reaction was effected on this total RNA, PCR was effected similarly as in Example 4-1(c) except for using the oligonucleotide shown by 5'-CTGAGCCTTAGAGCTCCAAG-3' (SEQ ID NO:69) as a sense primer and the oligonucleotide shown by 5'-GTGAAGCTTGAGTTTGAGGTTC-3' (SEQ ID NO:70) as an antisense primer, both chemically synthesized on the basis of the nucleotide sequence shown in SEQ ID NO:41. A portion of this PCR product was collected, and electrophoresed in a usual manner on 1%(w/v) agarose gel. Specific amplification of a DNA fragment was observed. This DNA fragment was sequenced similarly as in Example 2-1(a). This fragment comprised the nucleotide sequence shown in SEQ ID NO:41.

The paragraph bridging pages 37 and 38 has been amended as follows:

The amino acid sequence (SEQ ID NO:49) encoded by the nucleotide sequence shown in SEQ ID NO:41, aligned therewith, are compared with the partial amino acid sequences shown in SEQ ID NOs:24 to 31, determined in Example 3-2. These partial sequences were all included by the amino acid sequence aligned in SEQ ID NO:41 in the region from the 1st to 165th amino acids. In addition, the amino acid sequence of the IL-18-binding protein of human origin shown in SEQ ID NO:1 exhibited about 61% homology with the amino acid sequence aligned in SEQ ID NO:41 in the region from the 1st to 165th

amino acids. These facts suggested that the nucleotide sequence shown in SEQ ID NO:41 can encode the IL-18-binding protein of mouse origin by the region from the 235th to 729th nucleotides and that this IL-18-binding protein may have, as its whole sequence, the sequence from the first to 165th amino acids of the amino acid sequence aligned with this nucleotide sequence. The amino acid sequence thus suggested as that of the IL-18-binding protein of mouse origin and the nucleotide sequence encoding this are shown in SEQ ID NOs:2 and 33 separately.

The paragraph beginning at page 38, line 18 has been amended as follows:

A DNA capable of encoding the IL-18-binding protein of mouse origin, obtained by the method in Example 4-1(d), was placed in a 0.5-ml reaction tube in an amount of 1 ng, and this DNA was treated similarly as in Example 2-2(a) except for using the oligonucleotide shown by 5'-CTCGACGCCACCATGACCATGAGACACTGC-3' (SEQ ID NO:71) as a sense primer and the oligonucleotide shown by 5'-GCGGCCGCTCATTAGTGATGGTGATGGTGATGTGCAACCCCTGGGCCTGC-3' (SEQ ID NO:72), as an antisense primer, both on the basis of the nucleotide sequence shown in SEQ ID NO:33. Similarly as in Example 4-1(a), the PCR product was confirmed to contain the objective DNA fragment, and a plasmid vector inserted with this DNA fragment was obtained. This plasmid DNA was sequenced similarly as in Example 2-1(a). The plasmid DNA comprised the nucleotide sequence shown in SEQ ID NO:33.